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DOCUMENT-IDENTIFIER: US 6569633 B1
TITLE: Modulation of the sulfonylurea receptor and calcium in adipocytes for treatment of obesity/diabetes

Abstract Text (1):
The invention provides methods for identifying compounds and compositions useful in the regulation of weight, the treatment of obesity, diabetes and other insulin resistance-related disorders hypertension, cardiovascular disease and the like. The methods comprise the use of adipocytes and predipocytes in assays and screens for compounds or compositions of interest. The present invention recognizes the presence of the sulfonylurea receptor in adipocytes and its utility in identifying compounds and in treating obesity and other insulin resistance-related disorders.

Abstract Text (2):
In addition to assaying for agonists and antagonists of the sulfonylurea receptor, the methods of the invention also provide for identifying novel calcium channels or other calcium regulatory channels that are selectively expressed in human adipocytes as compared to human preadipocytes and for screening adipocytes for compounds that selectively antagonize calcium. These compounds may be used in the treatment of obesity and diabetes and other insulin resistance-related disorders.

Brief Summary Text (2):
This invention relates to methods of identification of the sulfonylurea receptor in human adipocytes and other adipocyte-selective calcium channels and calcium mobilization antagonists and use of these factors for treatment of obesity, diabetes, and insulin-resistance related syndromes.

Brief Summary Text (4):
Mice with dominant mutations at the agouti locus, including lethal yellow (A.sup.y) and viable yellow (A.sup.vy) are characterized by obesity, insulin resistance, and yellow coat color. The mouse agouti gene normally regulates differential pigment production in hair-bulb melanocytes. Each melanocyte in the hair bulb switches between the production of eumelanin (black) and phaeomelanin (yellow), producing the wild-type mouse coat color of a black hair with a subapical band of yellow. Agouti mutations disrupt this switching process and form a dominance hierarchy in which the dominant alleles produce a yellow coat and the recessive alleles produce a black coat.

Brief Summary Text (5):
Although agouti is normally expressed in neonatal skin, dominant agouti mutations are characterized by ectopic overexpression of agouti throughout life. This is due to mutations in the promoter/regulatory region rather than in protein-coding domain. Transgenic mice designed to express the agouti coding portion in a ubiquitous manner also develop a syndrome of obesity, hyperinsulinemia, hyperglycemia and yellow coat color, similar to A.sup.vy mutation, demonstrating that ectopic overexpression of the agouti gene is directly responsible for pleiotropic effects associated with dominant agouti mutations. However, the mechanism linking this pigmentation gene to obesity has not yet been identified.

Brief Summary Text (6):
There is needed methods for the identification of compositions and compounds for the treatment and control of insulin resistance-related disorders.

Brief Summary Text (8):
The invention provides methods for identifying compounds and compositions useful in the regulation of weight, the treatment of obesity, diabetes and other insulin resistance-related disorders hypertension, cardiovascular disease and the like. The methods comprise the use of adipocytes and predipocytes in assays and screens for compounds or compositions of interest. The present invention recognizes the presence of the sulfonylurea receptor in adipocytes and its utility in identifying compounds and in treating obesity and other insulin resistance-related disorders.

Brief Summary Text (9):
In addition to assaying for agonists and antagonists of the sulfonylurea receptor, the methods of the invention also provide for identifying novel calcium channels or other calcium regulatory channels that are selectively expressed in human adipocytes as compared to human preadipocytes and for screening adipocytes for compounds that selectively antagonize calcium. These compounds may be used in the treatment of obesity and diabetes and other insulin resistance-related disorders.

Brief Summary Text (10):
Once identified, the compounds of the invention can be used in pharmaceutical compositions for the treatment of insulin resistance-related disorders and to regulate lipogenesis and lipolysis.

Drawing Description Text (6):
FIG. 5. Multiplate well assay for the screening of compounds increasing [Ca.sup.2+].sub.i in human adipocytes. Adipocytes were prepared in a 96-well plate and loaded with fura-2-AM, 10 .mu.M as described for FIG. 2. All measurements were made in a Packard Fluorocount system using excitation filters of 340 and 380 nm and emission filter of 510 nm. The 340/380 ratio is proportional to [Ca.sup.2+].sub.i. The data shown represents four wells of a 96-well adipocyte plate. The data sets depicted by squares and diamonds received a pulse of KCl to stimulate Ca.sup.2+ influx, while the data sets depicted by circles and triangles received a pulse of vehicle at 5 seconds. At 10 seconds, all preparations are given a pulse of digitonin (25 .mu.M), which resulted in an expected increase in Ca.sup.2+ in all preparations, and at 14 seconds all preparations were given a pulse of pH 8.7 Tris/EGTA which results in a predictable decrease in [Ca.sup.2+].sub.i.

Detailed Description Text (2):
Methods are provided for the identification of compounds and compositions useful in the treatment of insulin resistance-related disorders. The methods of the invention use adipocytes and predipocytes in such assay methods. The method of the invention herein provides for the identification of a functional sulfonylurea receptor in adipocytes. The sulfonylurea receptor can be used to screen for agonists and antagonists of the receptor. The compounds find use in the treatment of obesity and diabetes. This method also provides for the method of identification of calcium channels or other calcium regulatory channels such as potassium ATP channels that are selective for human adipose tissue and/or human adipocytes. In particular, this method includes the production of human cultured adipocyte, isolation of human primary adipocytes, preparation of mRNA and/or cDNA libraries of said cells, and PCR cloning of novel channel/receptor cDNAs.

Detailed Description Text (4):
The invention also provides for the evaluation of these selective molecules in relevant obesity and diabetes animal models to determine the relevance of these molecules in treating human obesity and diabetes and insulin resistance-related disorders.

Detailed Description Text (5):
"Insulin resistance-related disorders" include obesity, diabetes, cardiac disorders, weight regulation disorders, eating disorders, anorexia, cachexia, and the like.

Detailed Description Text (6):
Thus, in one aspect, the invention provides a method of determining the ability of a compound to act as a SUR1 antagonist, comprising: (a) incubating cultured adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in

the presence and absence of said compound.

Detailed Description Text (9):
In another aspect, the invention provides a method of determining the ability of a compound to regulate lipogenesis or lipolysis, comprising: (a) incubating cultured adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound.

Detailed Description Text (10):
In yet another aspect, the invention provides a method of determining the ability of a compound to modulate obesity, diabetes, hypertension or other cardiovascular disease, comprising: (a) incubating cultured adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound.

Detailed Description Text (11):
In still another aspect, the invention provides a method of determining the ability of a compound to act as a potassium channel activator, comprising: (a) incubating cultured adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound.

Detailed Description Text (12):
In a variation of the above method, the cultured adipocytes are incubated with a SUR1 agonist in the presence and absence of said compound.

Detailed Description Text (13):
The invention further provides a method to determine the ability of a compound to act as a calcium channel antagonist, comprising: (a) incubating cultured adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said and adipocytes after incubation in the presence and absence of said compound.

Detailed Description Text (14):
In a further aspect, the invention provides a method of determining the ability of a compound to act as an adipocyte specific SUR1 antagonist, comprising: (a) incubating cultured preadipocytes and cultured adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said preadipocytes and adipocytes after incubation in the presence and absence of said compound.

Detailed Description Text (15):
The compounds and compositions of the invention include small molecules, large molecules, or mixtures thereof, peptides, polypeptides, antibodies, nucleotide sequences to control SUR gene expression including increasing or decreasing expression. Antisense molecules can be used to decrease expression and regulatory sequences can be used to design genes for increased expression.

Detailed Description Text (16):
The assays or biological screens of the invention provides a means for identifying and characterizing compounds that are agonists or antagonists of the sulfonylurea receptor, compounds that modulate intracellular calcium flux. The assays utilize adipocyte or preadipocyte cells. Thus, the methods of the invention allow for the identification of compounds using such cells for the treatment of insulin resistance-related disorders. Compositions and methods of the invention are also useful for the modulation of lipogenesis and lipolysis.

Detailed Description Text (17):
The compounds may be formulated into pharmaceutical compositions for administration. Such pharmaceutical compositions of the invention may include one or more protease inhibitors. An exemplary protease inhibitor is sodium pentosan polysulfate (PPS), a polysulfated polysaccharide.

Detailed Description Text (18):
The compositions of the invention may optionally include stabilizing agents including, but not limited to, amino acids (such as arginine, lysine, and glycine), sugars (such as sucrose, mannitol, and trehalose), salts (such as NaCl and MgCl.sub.2), surfactants, PEG, preservatives, antimicrobial agents, complexing agents (such as EDTA), and anti-oxidants.

Detailed Description Text (20):
By "isotonic" is meant a solution in which a cell will neither shrink nor swell. An example of an isotonic solution is 0.9% sodium chloride in water. Typically, an isotonic solution will have about the same osmotic pressure as the fluid phase of a subject's cells or tissue. However, a solution that is isosmotic with intracellular fluid will not be isotonic if it contains a solute that freely permeates cell membranes. To determine is a solution is isotonic, it is necessary to identify the concentration of solutes at which cells will retain their normal size and shape. Methods of determining the isotonicity of a solution are known to those skilled in the art. See, for example, Setnikar et al. (1959) JAPhA Sci Ed 48:628.

Detailed Description Text (21):
Those skilled in the art are familiar with a variety of pharmaceutically acceptable solutes useful in providing isotonicity in pharmaceutical compositions. Thus, the compositions of the invention further encompass components that can be used to provide isotonicity, for example, sodium chloride, glycine, mannitol, glycerol, sucrose, and other carbohydrates, acetic acid, other organic acids or their salts, and relatively minor amounts of citrates or phosphates. The ordinary skilled person would know of additional agents that are suitable for providing optimal tonicity.

Detailed Description Text (24):
Examples of such matrices include, but are not limited to, polyesters, copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al. (1983) Biopolymers 22:547-556), polyactides (U.S. Pat. No. 3,773,919 and EP 58,481), polyactate polyglycolate (PLGA), hydrogels (see, for example, Langer et al. (1981) J. Biomed. Mater. Res. 15:167-277; Langer (1982) Chem. Tech. 12:98-105), non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot.TM., and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Suitable microcapsules can also include hydroxymethyl cellulose or gelatin-microcapsules and poly-methylmethacylate microcapsules prepared by coacervation techniques or by interfacial polymerization. In addition, micro emulsions or colloidal drug delivery systems such as liposomes and albumin microspheres, may also be used. See Remington's Pharmaceutical Sciences (1990) (18.sup.th ed., Mack Pub. Co., Eaton, Pa.).

Detailed Description Text (25):
The compositions of the invention, comprising a pharmaceutically active agent and a succinate compound, can be stored for extended periods of time while maintaining the physical and biological integrity of the pharmaceutically active agent. Storage can be in liquid form or as a dried formulation, which can be reconstituted by adding liquid. When pharmaceutically active agents are proteins, storage can be facilitated by drying processes, such as lyophilization. Accordingly, the protein can be stored in the form of a freeze-dried composition. Thus, in one embodiment, the invention provides a lyophilized pharmaceutical composition comprising a succinate compound and a pharmaceutically active agent.

Detailed Description Text (32):
The compositions of the invention may be formulated with pharmaceutical carriers. Such carriers are generally known in the art. Suitable carriers for this invention are those conventionally used large stable macromolecules such as albumin, gelatin, collagen, polysaccharide, monosaccharides, polyvinyl-pyrrolidone, polylactic acid, polyglycolic acid, polymeric amino acids, fixed oils, ethyl oleate, liposomes, glucose, sucrose, lactose, mannose, dextrose, dextran, cellulose, mannitol, sorbitol, polyethylene glycol (PEG), and the like. Slow-release carriers, such as hyaluronic acid, may also be suitable. See particularly Prisell et al. (1992) Int. J. Pharmaceu. 85:51-56, and U.S. Pat. No. 5,166,331.

Detailed Description Text (33):

The pharmaceutical composition may additionally comprise a solubilizing agent or so-called solubility enhancer. Compounds containing a guanidinium group, most preferably arginine, are suitable solubility enhancers.

Detailed Description Text (36):
We have recently reported an increase in the expression of fatty acid synthase (FAS), a key enzyme in de novo fatty acid synthesis, in A.sup.vy /a mice compared to lean controls. Furthermore, we have also found recombinant agouti protein to stimulate both expression and activity of FAS and to increase triglyceride accumulation in 3T3-L1 adipocytes. Both basal and agonist-stimulated adipocyte lipolysis is impaired in agouti mutant mice compared to their wild-type littermates. This impairment may either be a consequence of the obesity syndrome or it may result from a direct inhibitory effect of agouti on adipocyte lipolysis. However, our recent data demonstrate that it is a direct effect of agouti, as follows. Short-term (1 hr) exposure of human adipocytes to recombinant agouti (100 nM) protein had no effect on basal lipolysis, although longer-term treatment (24 hr) caused a 60% decrease in basal lipolysis ($P<0.0001$). Short-term agouti treatment totally inhibited ACTH-induced lipolysis ($P<0.05$). Since melanocortin receptors (MCR) are involved in some actions of agouti, we next determined if agouti's anti-lipolytic effect is exerted through competitive antagonism of the ACTH receptor (MCR-2). 1 .mu.M forskolin, an adenylate cyclase activator, induced a 48% increase in lipolysis in human adipocytes ($P<0.05$); this effect was reversed by 100 nM agouti ($P<0.005$), demonstrating that the anti-lipolytic effect of agouti is distal to the ACTH receptor.

Detailed Description Text (37):
To test the physiological significance of adipose tissue-specific agouti expression in vivo, we generated a mouse line that models the human pattern of agouti in humans. These animals, like humans, express the agouti gene in adipose tissue and do not become obese or diabetic. However, when subjected to daily injections of insulin, they markedly increase their body weight compared with their non-transgenic insulin-treated controls. Similarly, these animals respond to a high sucrose diet with exaggerated weight gain compared to their non-transgenic sucrose-treated controls.

Detailed Description Text (39):
However, the actual mechanism of the action of agouti in the development of obesity is not clear. Agouti protein was recently demonstrated to inhibit melanogenesis independent of any melanocortin ligand. Moreover, we have shown that agouti regulates several cellular functions, such as Ca.sup.2+ signaling, independent of melanocortin receptor antagonism. Furthermore, we have demonstrated that agouti-induced obesity is not antagonized by the melanocortin receptor agonist, NDP-MSH.

Detailed Description Text (40):
Since [Ca.sup.2+]i has a key role in the pathogenesis of insulin resistance, obesity and hypertension, we have evaluated the role of the purified agouti gene product in regulating [Ca.sup.2+]i in cultured skeletal myocytes, vascular smooth muscle cells, and adipocytes. Agouti cDNA was subcloned into a baculovirus expression vector, packaged, and infected in T ni cells. The secreted peptide was collected and purified, with media collected from wild-type baculovirus-infected cells serving as controls. Purified agouti induced slow, sustained increases in [Ca.sup.2+]i in several cell types, including both murine and human adipocytes. Agouti protein also stimulated lipogenesis in both murine and human adipocytes via an apparently Ca.sup.2+ -dependent mechanism. Since the human homologue of agouti is expressed primarily in adipose tissue, and since [Ca.sup.2+]i plays an important role in the metabolic disorders of obesity and insulin resistance, agouti may similarly act via a paracrine mechanism on adipocytes Ca.sup.2+ signaling and thereby stimulate lipogenesis.

Detailed Description Text (41):
Intracellular Ca.sup.2+ ([Ca.sup.2+].sub.i) plays a key role in the metabolic disorders associated with obesity and insulin resistance (1-3). Obese patients exhibit increased basal [Ca.sup.2+]i in adipocytes, while increasing [Ca.sup.2+]i in rat adipocytes decreases insulin stimulated glucose transport. Further, Ca.sup.2+

channel blockade enhances insulin sensitivity in obese and glucose intolerant subjects. Sulfonylureas, such as glibenclamide, are insulin secretagogues widely used to stimulate insulin secretion in the treatment of non-insulin-dependent diabetes mellitus. Sulfonylureas depolarize pancreatic .beta. cells by blocking K.sub.ATP channels, thereby resulting in depolarization and secondary Ca.sup.2+ influx via L-type Ca.sup.2+ channels, which in turn triggers insulin release (8,9). The .beta. cell receptor for sulfonylureas, sulfonylurea receptor1 (SUR1), has been cloned (10). Recently, Alemzadeh et al reported that the drug diazoxide activates the .beta. cell K.sub.ATP channel and subsequently exerts an antiobesity effect in obese Zucker rats (11,12). Further, they recently reported that diazoxide exerted a significant antiobesity effect in hyperinsulinemic obese adults (13). However, this action was attributed to direct actions of diazoxide on .beta. cells, rather than direct effects of diazoxide on adipocyte metabolism. Patients treated with glibenclamide frequently experience weight gain as a side effect. These effects of glibenclamide and diazoxide on body weight have been attributed to their effect on circulating insulin rather than to any direct effect on adipocytes (11-13).

Detailed Description Text (42):
However, SUR agonists have previously been demonstrated to exert direct effects on adipocytes. Draznin et al (2,16) reported that glibenclamide increased [Ca.sup.2+].sub.i in isolated rat adipocytes in a dose dependent manner by promoting Ca.sup.2+ influx through voltage-dependent Ca.sup.2+ channels, while this effect was blocked by nitrendipine. Moreover, glibenclamide has been reported to potentiate peripheral insulin effects in isolated adipocytes (24,27). In contrast, Rajan et al (26) were unable to identify high affinity SUR in either isolated rat adipocytes or 3T3-L1 adipocytes. Moreover, they were unable to inhibit .sup.86 Rb.sup.+ efflux (a surrogate for K.sub.ATP channel activity) or increase [Ca.sup.2+].sub.i with glibenclamide. The reason for this discrepancy is not clear. However, several other investigators have reported both specific binding of sulfonylurea and specific post-receptor effects in murine and rat adipocytes. For example, rat adipocytes exhibit specific, saturable glibenclamide binding (K.sub.D of 1-3 .mu.M), which is displaced by other sulfonylureas, and sulfonylurea treatment of isolated rat adipocytes potentiates insulin receptor of glucose transport (24,25,27). Further, Muller et al (28,29) reported that glimepiride exhibits specific binding to 3T3-L1 and rat adipocytes, resulting in an insulin-mediated stimulation of glucose transport and non-oxidative glucose disposal. These effects were attributed to sulfonylurea-induced inhibition of cAMP level and protein kinase A activity. Thus, rodent adipocytes may exhibit SUR1 binding and functional response to this binding. However, other data cited above argues that this may not be true. Thus, prior to the present invention there was clearly considerable controversy in this area.

Detailed Description Text (46):
Adipocyte SUR1 also regulated lipogenesis, as demonstrated by human adipocytes treated with SUR1 agonist and antagonist. FAS and GPDH activities are commonly used lipogenic markers, although other markers of lipogenesis will most likely give similar results. Cells treated with a SUR agonist such as glibenclamide caused increased FAS activity between 50%-500% from basal levels, which may be completely blocked by 10 .mu.M diazoxide, a K.sub.ATP channel activator or other K.sub.ATP channel activator s such as pinacidil, cromakalin, nicorandil, aprikalim, and partially inhibited by nitrendipine, an L-type Ca.sup.2+ channel antagonist or similar L-type Ca.sup.2+ channel antagonists characterized by examples from these three classes of compounds: Phenylalkylamines (example, verapamil); Benzothiazepines (example, diltiazem); and dihydropyridines (examples, nitrendipine, nifedipine, amlodipine, felodipine, isradipine, nicardipine, nisoldipine. Similarly, SUR agonists such as glibenclamide stimulated increased GPDH activity between 50-100%, which may be completely blocked by 10 .mu.M diazoxide, a K.sub.ATP channel activator, and partially inhibited by nitrendipine, an L-type Ca.sup.2+ channel antagonist.

Detailed Description Text (48):
Accordingly, using the above techniques and human adipocytes, SUR antagonists that can decrease the lipid accumulation in adipocytes can be identified. Adipocytes were prepared as described in example 3 or as described in U.S. Pat. No. 6,153,432 and cultured as described in these references. Compounds may then be added to the cells and compounds inhibiting glibenclamide-mediated increases may be SUR antagonists,

such as nitrendipine. Other methods to determine lipogenesis include determination of total lipid accumulation or the measurement of other lipogenic enzyme activities such as diglyceride acyltransferase. A correlation of calcium mobilization with the biochemical effects observed with sulfonylurea reagents is also clear. Another method to identify SUR antagonists is to use calcium measurements to find SUR antagonists. Using the same methodology described above, one skilled in the art can identify SUR antagonists for human adipocytes.

Detailed Description Text (49):
SUR regulates intracellular calcium after binding of an agonist by inhibiting an inwardly rectifying potassium channel, the K.sup.+ -ATP channel. Accordingly, various compounds, such as diazoxide, that stimulate these channels will effectively block calcium accumulation. Glibenclamide and diazoxide serve as an antagonistic pair in the pancreatic .beta.-cell, the former increasing intracellular Ca.sup.2+ -stimulated insulin release and the latter inhibiting this process. Incubation of adipocytes with diazoxide prevented increase intracellular calcium in response to sulfonylureas such that there was no detectable response to any dose of glibenclamide in the presence of 2-10 .mu.M diazoxide. This blockade of glibenclamide-induced intracellular calcium signaling also prevented sulfonylurea-induced increases in fatty acid synthase activity and triglyceride. Accordingly, a method to identify such potassium channel activators would involve the use of human adipocytes prepared as described above. The cells are incubated with 1-20 .mu.M glibenclamide as a typical SUR agonist, although others such as glipizide, gliclazide, tobutamide, glimepiride, or chloropamide could be used. The treated cells are then incubated with compounds to determine their ability to inhibit the increase in [Ca.sup.2+].sub.i caused by the SUR agonist as described above. Additionally, measurement of lipogenesis or lipolysis response may also be used as described above.

Detailed Description Text (50):
Calcium channel antagonist treatment has been shown to reverse agouti-induced obesity and insulin resistance in mice. Accordingly, the following method is described to identify calcium channel blockers that can cause weight loss. Common obesity models that can be used in this method include transgenic agouti mice, yellow mice, ob/ob mice, db/db mice, or Zucker rats. Animals are maintained on standard lab chow diets. When significant adiposity is apparent, animals are then treated with the compounds in the appropriate vehicles. Weight loss and food intake was monitored over the course of 1-8 weeks. Fat pad weight can be determined as an endpoint for weight loss. Also, FAS and GPDH activity can be determined in treated and vehicle-treated animals.

Detailed Description Text (51):
The ability to discover novel SUR antagonists and calcium channel blockers would be facilitated by the use of a facile and reproducible assay using human adipocytes as the target cell type. Use of another cell type, such as HEK293 cells, would allow characterization of the adipocyte-selectivity of novel compounds. Accordingly, the following method describes the use of human adipocytes in a screening assay for SUR antagonists and calcium channel blockers.

Detailed Description Text (52):
Human subcutaneous abdominal adipose tissue was obtained from patients undergoing elective abdominal cosmetic surgery, although other types of adipose tissue, such as mesenteric, retroperitoneal, perirenal, or omental may be useful in identifying these compounds. Preadipocytes are isolated by collagenase treatment of the tissue and cultured adipocytes prepared as described (patent filed; Jan. 29, 1999; Ser. No. 09/240,029). Agents that regulate calcium flux in adipocytes as noted above can mediate lipolysis. Human adipocytes may be cultured in multi-well plates with different treatments between 1 hour or 24 hours as indicated, and glycerol release into the culture medium was determined as an indicator for lipolysis using a one-step enzymatic fluorometric method. The media collected from 1 hr treatments were used directly for glycerol measurements. For 24 hr treatments, media were removed, and cells were washed with Hank's Balanced Salt Solution and incubated in fresh medium for a further 10-60 min. This media were then used for glycerol measurements for 24 hr treatments. After medium was collected, cells were processed for total protein concentration by a modified Bradford method using Coomassie Blue

dye (PIERCE, Rockford, Ill.) or other comparable protein determination methods to allow correction for differences in cell density/well.

Detailed Description Text (53):
Correlation of lipolysis with flux of intracellular calcium ([Ca.sup.2+]i) concentration is then performed. Human cultured adipocytes are plated as described above and compounds added for a defined period of time 30-120 minutes. [Ca.sup.2+]i levels are then measured fluorometrically using dual excitation (340 and 380 nm)/single emission (510 nm) fluorometry and [Ca.sup.2+]i is calculated by the computer in the fluorometer. As a control, after loading fura-2/AM cells are pretreated with nitrendipine (RBI, Natick, Mass.) (30 .mu.M, final concentration) for 10 min at 37.degree. C.

Detailed Description Text (54):
Selectivity of the compounds may then be determined by analysis of intracellular calcium concentrations in a variety of relevant cell types such as 3T3-L1 adipocytes, primary human adipocytes, differentiated cultured adipocytes, smooth muscle cells, pancreatic beta cells, cardiomyocytes and other cell types. Adherent cell types are plated in a multi-well plate and [Ca.sup.2+]i levels are then measured fluorometrically using dual excitation (340 and 380 nm)/single emission (510 nm) fluorometry as described above. As a control, after loading fura-2/AM cells are pretreated with nitrendipine (RBI, Natick, Mass.) (30 .mu.M, final concentration) for 10 min at 37.degree. C.

Detailed Description Text (57):
Methods of the invention are useful for identifying novel sulfonylurea receptor antagonist and/or calcium channel blockers that are selective for adipocytes. Such compounds will be useful in the treatment and control of obesity, diabetes, and other insulin related disorders. Using the methods of the invention, the following information can be obtained: The presence of a novel K.sup.+ -ATP channel and/or sulfonylurea receptor that will mediate intracellular calcium flux and have therapeutic utility in the treatment of diabetes, obesity or cardiovascular disease; The use of known sulfonylurea receptor and/or calcium channel antagonists that will mediate intracellular calcium flux for therapeutic utility in the treatment of obesity; The use of any adipocyte-specific calcium mobilizing agent for the treatment of obesity; The use of adipocytes (human or murine) to screen for the above described calcium mobilizing agents using similar methodology to that described in the patent; The presence of a novel sulfonylurea receptor and/or calcium channel in the adipocyte that will mediate intracellular calcium flux and have therapeutic utility in the treatment of obesity; The use of the above described channels for the screening and identification of compounds that have therapeutic utility in the treatment of obesity.

Detailed Description Text (67):
Compounds that Modulate SUR Activity in Adipocytes Mediates Biochemical Parameters that Influence Obesity and Diabetes

Detailed Description Text (68):
Human subcutaneous abdominal adipocytes were isolated from healthy patients undergoing cosmetic liposuction using methodology we have previously described. Briefly, the tissue was washed several times with Hank's Balanced Salt Solution, minced into small fragments and digested with type I collagenase (1 mg/mL) in a shaking water bath at 37.degree. C. for 30-40 minutes. Cells were then filtered through a sterile nylon filter (500 .mu.m mesh) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% fetal bovine serum, 1% bovine serum albumin, 100 U/mL penicillin, 100 .mu.g/mL streptomycin and 50 .mu.g/mL gentamicin. Cells were subsequently cultured in suspension in sterile polypropylene flasks in a humidified 37.degree. C./5% CO.sub.2 incubator. After one day, suspended (floating) adipocytes were transferred to fresh medium and maintained as a thin layer at the top of the culture medium to ensure access to nutrients. The cells were maintained for another 24 hours in this medium and then changed to fresh medium with the addition of SUR agonists (such as glibenclamide) for up to 48 hours with the compounds to be tested. Medium was changed every 24 hours, and cell viability was verified with trypan blue exclusion at the end of representative experiments.

Detailed Description Text (69):
Exposure of human adipocytes to graded doses of the sulfonylurea, glibenclamide, resulted in slow, sustained increases in intracellular free calcium levels. The maximum response is 388.+-.72 nM over a baseline of 190 nM for human adipocytes. For human adipocytes, the EC.sub.50 is approximately 1 .mu.M. This sulfonylurea-mediated increase in adipocyte intracellular calcium is linked to increases in human adipocyte fatty acid synthase activity. Glibenclamide (2 .mu.M) increased fatty acid synthase activity from 1.93.+-.0.24 to 7.90.+-.0.4 nmol/min/mg protein. A further increase, to 12.8.+-.0.6 nmol/min/mg was noted with 10 .mu.M glibenclamide ($p<0.001$). This resulted in a dose-responsive stimulation of triglyceride accumulation, from 28.2.+-.4.5 to 46.1.+-.6.3 to 58.0.+-.3.4 .mu.g/mg protein.

Detailed Description Text (70):
To prove a correlation of calcium mobilization with the biochemical effects observed with sulfonylurea reagents, the effect of calcium channel antagonists on the sulfonylurea mediated triglyceride accumulation was examined. First, the dihydropyridine calcium channel antagonist, nitrendipine, completely blocked the sulfonylurea mediated increases in adipocyte intracellular calcium levels. This block of intracellular calcium results in inhibition of increased fatty acid synthase activity and triglyceride accumulation in response to sulfonylureas. The aforementioned increase in increased fatty acid synthase activity from 1.93.+-.0.24 to 7.90.+-.0.4 nmol/min/mg protein in response to 2 .mu.M glibenclamide was decreased to 2.12.+-.0.0.43 nmol/min/mg protein, which was not significantly different from control, when 30 .mu.M nitrendipine was included.

Detailed Description Text (72):
Compounds that Modulate Intracellular Calcium Concentration in Adipocytes Mediate Biochemical Parameters that Influence Obesity and Diabetes

Detailed Description Text (73):
Adipocytes exposed to KCl (40 mM, to depolarize and activate voltage gated Ca.sup.2+ channels) for two hours and then harvested approximately 72 hours later exhibited a two-fold increase in fatty acid synthase activity, from 3.93.+-.0.11 to 6.24.+-.0.2 nmol NADPH/min/mg protein ($p<0.01$). Similarly, stimulation of receptor-mediated Ca.sup.2+ influx with either parathyroid hormone (PTH; 4.8 nM) or arginine vasopressin (AVP; 1 nM) also resulted in a two-fold increase in fatty acid synthase activity, to 6.9.+-.0.1 nmol/min/mg protein ($p<0.01$). Exposure to these Ca.sup.2+ agonists also inhibited lipolysis. Twenty-four hour exposure to mild depolarization with KCl (10 mM) resulted in a decrease in lipolysis to 75.9.+-.4.1% of control levels ($p<0.005$). Short-term (one-hour) experiments with more complete depolarization demonstrate that depolarizing human adipocytes with 25 and 40 mM KCl inhibited forskolin-induced lipolysis by 42.+-.5 and 50.+-.1.7%, respectively ($p<0.05$). Similarly, short-term treatment with 1 nM AVP caused a 56.+-.6.6% inhibition of forskolin-induced lipolysis ($p<0.0$).

Detailed Description Text (74):
Stimulation of fatty acid synthase and concomitant inhibition of lipolysis results in increased lipid accumulation in human adipocytes. Adipocytes were treated as described above, harvested, and their triglyceride content was analyzed and normalized for protein content. Treatment with KCl (40 mM) or PTH (4.8 nM) resulted in significant increases in triglyceride content, from 30.3.+-.0.3 (control) to 43.5.+-.0.5 (KCl) and 54.5.+-.0.6 (PTH) .mu.g/mg protein ($p<0.01$). Similarly, a two-hour exposure to 10 nM of the Ca.sup.2+ ionophore, A23187, resulted in a marked increase in triglyceride accumulation over the ensuing 48 hours, from 31.4.+-.2.4 to 52.+-.7 .mu.g/mg protein ($p<0.001$). Thus, these calcium-stimulated biochemical activities will contribute to the development of adipocyte hypertrophy and potentially obesity.

Detailed Description Text (75):
Calcium channel antagonists do the opposite of that described above. Use of non-selective calcium channel antagonists will inhibit fatty acid synthase activity, stimulate lipolysis and decrease triglyceride accumulation in calcium-stimulated human adipocytes. For example, 30 .mu.M nitrendipine resulted in complete inhibition of each of the calcium-stimulated activities noted above. Moreover, the well-known activation of lipogenesis by insulin (1 nM) was completely inhibited by

nitrendipine. Insulin increased fatty acid synthase activity from 3.95.+-.0.11 to 14.56.+-.0.22 nmol/min/mg protein ($p<0.0001$), while inclusion of nitrendipine (30 .mu.M) completely prevented this increase (4.12.+-.0.03 nmol/min/mg). Similarly, inclusion of nitrendipine caused a 40% reduction in insulin-stimulated triglyceride accumulation ($p<0.001$).

Detailed Description Text (76):
Glibenclamide regulates intracellular calcium by inhibiting an inwardly rectifying potassium channel, the K.sup.+ -ATP channel. This results in depolarization and consequent activation of voltage-gated Ca.sup.2+ channels. Accordingly, various compounds, such as diazoxide, that stimulate these channels will effectively block calcium accumulation. Glibenclamide and diazoxide serve as an antagonistic pair in the pancreatic .beta.-cell, the former increasing intracellular Ca.sup.2+ -stimulated insulin release and the latter inhibiting this process. Since we found glibenclamide to stimulate adipocyte intracellular Ca.sup.2+ despite there having been no previous report of adipocytes expressing any type of sulfonylurea receptor, we examined whether diazoxide would antagonize this effect. Incubation of adipocytes with diazoxide prevented increase intracellular calcium in response to sulfonylureas such that there was no detectable response to any dose of glibenclamide in the presence of 5 .mu.M diazoxide. Accordingly, this blockade of glibenclamide-induced intracellular calcium signaling also prevented sulfonylurea-induced increases in fatty acid synthase activity and triglyceride. The aforementioned increases in fatty acid synthase activity, from 1.93.+-.0.24 (control) to 7.90.+-.0.4 (2 .mu.M glibenclamide) to 12.8.+-.0.6 (10 .mu.M glibenclamide) nmol/min/mg protein was completely prevented by inclusion of 5 .mu.M diazoxide. Fatty acid synthase activity in the presence of diazoxide and 2 or 10 .mu.M glibenclamide was 1.00.+-.0.10 and 1.21.+-.0.31, respectively; these values were not significantly different from control (1.93.+-.0.26). Further, as an apparent consequence of hyperpolarizing the adipocytes, diazoxide (5 .mu.M) also inhibited the lipogenic response to 40 mM KCl, as reflected in an inhibition of KCl-activated fatty acid synthase activity. Fatty acid synthase activity was increased from 2.87 (control) to 6.20.+-.0.57 nmol/min/mg protein in response to 40 mM KCl ($p<0.01$). This response was decreased to 3.24.+-.0.41 nmol/min/mg protein (not significantly different from control) in the presence of 5 .mu.M diazoxide. Similarly, fatty acid synthase activity in the presence of 1 nM insulin was 14.56.+-.0.22 nmol/min/mg protein and was reduced 7.08.+-.0.10 nmol/min/mg in the presence of 1 nM insulin and 5 .mu.M diazoxide.

Detailed Description Text (82):
Method for Screening Compounds that Affect Calcium Levels in Human Adipocytes

Detailed Description Text (83):
Human subcutaneous abdominal adipose tissue was obtained from patients undergoing elective abdominal cosmetic surgery. These patients were normal, with no known history of metabolic disorders. Isolated adipocytes were prepared from human adipose tissue by methods available in the art. Briefly, adipose tissue was washed with Hanks solution, roughly chopped with scissors and digested in Hanks solution containing collagenase type 1 (1 mg/ml) for 1 hr at 37.degree. C. Collagenase was washed out with HEPES buffered salt solution (HBSS) containing 138 mM NaCl, 1.8 mM CaCl.sub.2, 0.8 mM MgSO.sub.4, 0.9 mM NaH.sub.2 PO.sub.4, 4 mM NaHCO.sub.3, 25 mM glucose, 6 mM glutamine, 20 mM HEPES, 0.5% bovine serum albumin at the end of digestion. Cells were then filtered through a sterile nylon filter (500 .mu.m mesh), and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% fetal bovine serum (FBS), 1% bovine serum albumin (BSA), 100 U/ml penicillin, 100 .mu.g/ml streptomycin and 50 .mu.g/ml gentamicin.

Detailed Description Text (84):
Agents that regulate calcium flux in adipocytes as noted above can mediate lipolysis. Human adipocytes (1 mL cells/well) were incubated in 24-well plates with different treatments for either 1 hour or 24 hours as indicated, and glycerol release into the culture medium was determined as an indicator for lipolysis using a one-step enzymatic fluorometric method. The media collected from 1 hr treatments were used directly for glycerol measurements. For 24 hr treatments, media were removed, and cells were washed with Hank's Balanced Salt Solution and incubated in fresh medium for a further 30 min. This media were then used for glycerol measurements for 24 hr treatments. After medium was collected, HClO.sub.4 was added

to give a final concentration of 3% for deproteinization. The sample was centrifuged at 9000.times.g for 20 min. The supernatant was then collected and neutralized with 10N NaOH. Samples were stored at -80.degree. C. prior to glycerol assay. Cells were collected, homogenized and stored at -80.degree. C. for subsequent total protein correction by a modified Bradford method using Coomassie Blue dye (PIERCE, Rockford, Ill.).

Detailed Description Text (86):
Selectivity of the compounds may then be determined by analysis of intracellular calcium concentrations in a variety of relevant cell types. [Ca.sup.2+]i was measured in L6, A7r5 and HEK-293 cells in 3T3-L1 cells. Monolayers of non-fused L6 cells and confluent A7r5 cells after 14-16 hrs starvation in a serum free culture media were rinsed with Hanks solution and detached by incubation in 2 ml of trypsin (0.05%) for 2-3 min at 37.degree. C. and released by pipetting with culture media containing serum. Confluent HEK-293 cells after 3-4 hrs starvation in a serum free culture media were released just by pipetting without trypsinization. Cells were harvested by centrifugation at 50.times.g, and resuspended in 1 ml of HBSS at a density of approximately 10.sup.6 cells/ml. For 3T3-L1 cells, monolayers of differentiated adipocytes were rinsed with serum-free DMEM and exposed to 0.05% trypsin for 3 min. The solution was decanted, and the cells were incubated for 3 min in culture media. Cells were detached by pipetting and collected by centrifugation at 50.times.g. Cells were then incubated in culture media to recover for 1 hr followed by incubation in serum free DMEM for 4 hrs at 37.degree. C. in an atmosphere of 5% CO.sub.2, and collected by centrifugation and resuspended in HBSS at a density of approximately 10.sup.6 cells/ml. Cell suspensions were chilled in ice for 5 min and loaded with 10 mM fura-2/AM (final concentration) (Sigma, St Louis, Mo.) in the dark for 20 min at 37.degree. C. For 3T3-L1 adipocytes chilling step was skipped. For the [Ca.sup.2+]i measurements L6, A7r5, HEK-293 cells and 3T3-L1 cells were resuspended in 1 ml of HBSS and transferred to a 1-ml, 37.degree. C. cuvette and primary adipocytes were resuspended in 3-5 ml of HBSS at the concentration of .about.20.times.10.sup.4 cells/ ml and 2.4 ml of cell suspension were transferred to a 3-ml, 37.degree. C. cuvette. [Ca.sup.2+]i levels were then measured fluorometrically in suspensions using dual excitation (340 and 380 nm)/ single emission (510 nm) fluorometry and [Ca.sup.2+]i was then calculated by the computer in the fluorometer.

Detailed Description Text (91):
Determination of Adipocyte Selective Calcium Antagonist Compounds Utility in Treating Obesity/diabetes

Detailed Description Text (92):
Use of a transgenic model of obesity and insulin resistance is described. It should be noted that most genetic and diet-induced models of obesity/diabetes would be suitable for this purpose.

Detailed Description Text (94):
Prior to being placed on the experimental diets, 7-week-old male transgenic and control mice were acclimatized on a powdered high-fat diet (Mouse Diet 5015, PMI Feeds, >11% fat) for 1 week. They were randomly assigned to either a control or nifedipine (Sigma, St. Louis, Mo.) (1 g/kg diet) diet and were fed ad libitum for 30 days. Food intakes were measured every other day. On day 27, food was held overnight for 12 hr fasting and blood was collected from the tail vein for glucose determination, followed by refeeding. On day 30, animals were anesthetized with sodium pentobarbital (50 mg/kg body weight) (Abbott Lab. North Chicago, Ill.) and blood was obtained by cardiac puncture for blood glucose and plasma triglyceride and insulin determination. Fat pads (epididymal, perirenal, retroperitoneal, inguinal, and subscapular fats) were dissected, immediately weighed, frozen in liquid nitrogen, and stored at -80.degree. C. FAS activity was measured in adipose tissues as described below. Blood glucose was measured using blood glucose monitoring system (Milpitas, Calif.). Plasma triglyceride levels were measured spectrophotometrically using an enzyme-based assay kit (Sigma, St. Louis, Mo.), and plasma insulin levels were measured by radioimmunoassay kit (INCSTAR, Stillwater, Minn.). Fatty acid synthase activity was measured by a modification of the spectrophotometric method. Subcutaneous adipose tissue were sonicated (1:3 wt/vol) in 250 mM sucrose buffer containing 1 mM EDTA (Gibco, Gaithersburg, Md.), 1 mM dithiothreitol and 100 .mu.M

phenylmethylsulfonyl fluoride (Sigma, St. Louis, Mo.) (pH 7.4). Homogenates were centrifuged at 14,000.times.g for 15 min (at 4.degree. C.), and the supernatants were used for enzyme assays. Assays were started by the addition of malonyl CoA, and enzyme activities were expressed as nmol NADPH oxidized/min/mg of protein. Protein was determined by the modified method of Lowry using bovine serum albumin as a standard. All data were presented as mean.+-.SE for four groups of mice; control mice on control diet, control mice on nifedipine diet, transgenic mice on control diet, and transgenic mice on nifedipine diet. Data were analyzed via two-way (diet.times.animal) analysis of variance or, in cases where only two groups were being compared, by Student's t-test.

Detailed Description Text (95):
At study initiation (8 weeks of age), transgenic mice ubiquitously expressing agouti (n=12) exhibited significantly higher body weight than the control mice (n=10) (30.54.+-.0.66 vs. 27.26.+-.0.28 g; $p<0.001$). The increased body weight of the transgenic mice was maintained over the 30 days on diet, and nifedipine was without effect in both groups. The transgenic mice also exhibited an approximately 2-fold increase in fat pad mass (both visceral and subcutaneous) compared to the control mice (2.62.+-.0.27 vs. 1.21.+-.0.19 g; $p=0.002$). However, nifedipine treatment resulted in a significant reduction in fat pad weight, compared to control diet (1.81.+-.0.14 vs. 1.58.+-.0.23 g, $p<0.001$ in visceral [epidydimal, perirenal and retroperitoneal] fat; 0.81.+-.0.14 vs. 0.62.+-.0.1, $p=0.02$ in subcutaneous [inguinal and subscapular] fat) in the transgenic mice, but was without effect in the control mice. The total weight of the five fat pads measured was decreased by 18% in the nifedipine-treated transgenic animals ($p<0.007$). Gastrocnemius muscle weight was not different between control and transgenic mice however, nifedipine treatment significantly increased gastrocnemius muscle weight by 12% compared to the control diet (0.284.+-.0.005 vs. 0.253.+-.0.005 g; $p=0.0009$) in the transgenic mice, and was without effect in the control mice. This combination of reduced fat pad mass and increased muscle mass may explain the lack of nifedipine effect on body weight in the transgenic mice. To test the role of de novo fatty acid synthesis in agouti-associated obesity, we measured the activity of FAS in adipose tissue. The transgenic mice exhibited a 7.2-fold increase in FAS activity in subcutaneous adipose tissue compared to the control mice, while nifedipine treatment completely prevented this stimulation of FAS. The transgenic mice had approximately 2-fold higher fed plasma insulin levels than the control mice ($p<0.05$) (Table 1). Nifedipine treatment completely blocked the hyperinsulinemia in the transgenic mice, but was without effect on the control mice (Table 1). Nifedipine treatment also improved insulin sensitivity, as manifested by the fall in plasma insulin to glucose ratio in the transgenic mice ($p<0.05$) (Table 1). Nifedipine treatment was without effect on blood glucose levels in either fasted or fed mice. There was also no effect of nifedipine on fasted plasma triglyceride contents (Table 1).

Detailed Description Text (96):
The following references are herein incorporated by reference: Michaud et al., J Endocrinol. November 1997; 155(2): 207-209; Kim et al., Am J Physiol. March 1997; 272(3 Pt 1): E379-E384; Mynatt et al., Proc Natl Acad Sci U S A. Feb. 4, 1997; 94(3): 919-922; Kim et al., FASEB J. December 1996; 10(14): 1646-1652; Maher et al., J Nutr. October 1996; 126(10): 2487-2493; Raynor et al., J Am Diet Assoc. September 1996; 96(9): 854; Jones et al., Am J Physiol. July 1996; 271(1 Pt 1): E44-E49; Jones et al., Am J Physiol, January 1996; 270(1 Pt 1): E192-E196; Maher et al., J Nutr. October 1995; 125(10): 2618-2622; Kaplan et al., J Pediatr. August 1995; 127(2): 200-205; Zemel M B, J Nutr. June 1995; 125(6 Suppl): 1715S-1743S; Zemel M B, J Nutr. June 1995; 125(6 Suppl): 1715S-1717S; Zemel M B et al., Proc Natl Acad Sci USA. May 23, 1995; 92(11): 4733-4737; Kim et al., J Nutr. May 1994; 124(5): 713-716; Abel et al., Am J Hypertens. June 1993; 6(6 Pt 1): 500-504; Zemel et al., Am J Physiol. March 1992; 262(3 Pt 1): E368-E371; Ambrozy et al., Am J Hypertens. July 1991; 4(7 Pt 1): 592-596; Sowers et al., Am J Hypertens. July 1991; 4(7 Pt 2): 466S-472S; Zemel et al., Am J Hypertens. June 1991; 4(6): 537-539. 1. Byyny, R. L., Loverde, M., Llotd, S., Mitchell, W., and Draznin, B. (1992) Cytosolic Calcium and insulin resistance in elderly patients with essential hypertension. Am.J. Hypertension.5, 459-464 2. Draznin, B., Sussman, K. E., Eckel, R. H., Kao, M., Yost, T. and Sherman, N. A. (1988) Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. J. Clin Invest 82, 1848-1852 3. Draznin, B., Sussman, K., Kao, K., Lewis, D. and Sherman, N. (1987) The existence of

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Detailed Description Paragraph Table (1):
TABLE 1 Blood Glucose and Plasma Insulin and Triglyceride Levels Animal Control Transgenic Treatment Control Nifedipine Control Nifedipine Glucose (mg/dl) (fasted) 121.3 .+-. 6.5 125.3 .+-. 7.8 115.5 .+-. 8.9 100.4 .+-. 15.2 (fed) 243.0 .+-. 7.6 213.8 .+-. 18.6 230.6 .+-. 21.6 235.8 .+-. 9.7 Insulin 14.96 .+-. 5.3 16.74 .+-. 8.69 32.39 .+-. 4.9* 13.40 .+-. 1.96 (ng/ml) (fed) Insulin/ 0.06 .+-. 0.02 0.084 .+-. 0.05 0.140 .+-. 0.02* 0.057 .+-. 0.01 Glucose (fed) Tri- 107 .+-. 14.2 83.4 .+-. 20.1 76.5 .+-. 11.6 62.8 .+-. 5.6 glyceride (mg/dl) (fasted) Values are the mean .+-. SE. Data were compared in a row. $p < 0.05$

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CLAIMS:

1. A method of determining the ability of a compound to modulate obesity by acting as a sulfonylurea-1 (SUR 1) potassium channel activator, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate obesity by acting as a SUR 1 potassium channel activator.

2. The method of claim 1, wherein the lipogenic marker is selected from the group consisting of: Ca.sup.2+ influx, fatty acid synthase (FAS) activity, glycerol phosphate dehydrogenase (GPDH) activity and triglyceride accumulation.

4. The method of claim 1, wherein said cultured adipocytes are incubated with an SUR 1 agonist in the presence and absence of said compound.

5. A method of determining the ability of a compound to modulate obesity by acting as an adipocyte potassium channel activator, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate obesity by acting as an adipocyte potassium channel activator.

8. The method of claim 5, wherein said cultured adipocytes are incubated with an SUR 1 agonist in the presence and absence of said compound.

9. A method of determining the ability of a compound to modulate obesity by acting as an SUR 1 antagonist, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate obesity by acting as an SUR 1 antagonist.

12. A method of determining the ability of a compound to modulate obesity by acting as an adipocyte specific SUR 1 antagonist, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate obesity by acting as an adipocyte specific SUR 1 antagonist.

15. A method of determining the ability of a compound to modulate diabetes by acting as a sulfonylurea-1 (SUR 1) potassium channel activator, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate diabetes by acting as a SUR 1 potassium channel activator.

16. The method of claim 15, wherein said cultured adipocytes are incubated with a SUR 1 agonist in the presence and absence of said compound.

17. A method of determining the ability of a compound to modulate diabetes by acting as an adipocyte potassium channel activator, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate diabetes by acting as an adipocyte potassium channel activator.

20. The method of claim 17, wherein said cultured adipocytes are incubated with SUR 1 agonist in the presence and absence of said compound.

21. A method of determining the ability of a compound to modulate diabetes by acting as an SUR 1 antagonist, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate diabetes by acting as an SUR 1 antagonist.

24. A method of determining the ability of a compound to modulate diabetes by acting as an adipocyte specific SUR 1 antagonist, comprising: (a) incubating cultured human adipocytes in the presence and absence of said compound, and (b) comparing the level of at least one lipogenic or anti-lipolytic marker in said adipocytes after incubation in the presence and absence of said compound, wherein a difference in the level of said marker is correlated with the ability of the compound to modulate diabetes by acting as an adipocyte specific SUR 1 antagonist.

	Beasley, Ellen M., Darnestown, MD, United States
PATENT ASSIGNEE(S):	PE Corporation (NY), Norwalk, CT, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6479270	B1	20021112
APPLICATION INFO.:	US 2000-685853		20001011 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-182194P	20000214 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Achutamurthy, Ponnathapu
ASSISTANT EXAMINER:	Swope, Sheridan L.
LEGAL REPRESENTATIVE:	Celera Genomics, Sun-Hoffman, Lin
NUMBER OF CLAIMS:	8
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	3 Drawing Figure(s); 33 Drawing Page(s)
LINE COUNT:	3720

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 6 OF 25 USPATFULL

TI **Receptor** derived peptides as modulators of **receptor** activity

AB Oligopeptides having an amino acid sequence corresponding to a **receptor**'s extracellular domain, and having sequence similarity to regulatory peptides from MHC class I antigens, enhance or replace the physiological response of ligand **binding** to the corresponding **receptor**. The oligopeptides are used in diagnosis and therapy of diseases that involve inadequate or inappropriate **receptor** response as well as in the screening of drug candidates that affect surface expression of receptors. Also useful for drug screening is a modified **receptor** molecule, where the sequence corresponding to the regulatory peptide is modified or deleted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:287154 USPATFULL
TITLE:	**Receptor** derived peptides as modulators of **receptor** activity
INVENTOR(S):	Olsson, Lennart, Mountain View, CA, UNITED STATES Naranda, Tatjana, Mountain View, CANADA

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002160013	A1	20021031
APPLICATION INFO.:	US 2001-991548	A1	20011120 (9)

RELATED APPLN. INFO.:	Continuation of Ser. No. US 1998-28937, filed on 24 Feb 1998, GRANTED, Pat. No. US 6333031 Continuation of Ser. No. US 1997-788820, filed on 23 Jan 1997, GRANTED, Pat. No. US 6346390 Continuation of Ser. No. US 1996-701382, filed on 22 Aug 1996, GRANTED, Pat. No. US 6004758 Continuation of Ser. No. US 1996-612999, filed on 8 Mar 1996, GRANTED, Pat. No. US 5952293
DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	MORRISON & FOERSTER LLP, 755 PAGE MILL RD, PALO ALTO, CA, 94304-1018
NUMBER OF CLAIMS:	36
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	20 Drawing Page(s)
LINE COUNT:	2231

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 7 OF 25 USPATFULL
TI Glycogen synthase kinase-3 inhibitors
AB Peptide inhibitors of glycogen synthase kinase-3 (GSK-3) have an amino acid sequence motif of XZXXXS(p)X, wherein S(p)=phosphorylated serine or phosphorylated threonine, X=any amino acid, and Z=any amino acid except serine or threonine. These inhibitors, which are about 7 to 50 amino acids long, are specific for GSK-3 and strongly inhibit the enzyme with an IC.sub.50 of about 150 .mu.M. Also provided are methods of treating biological conditions mediated by GSK-3 activity, such as potentiating **insulin** signaling in a subject, treating or preventing type 2 diabetes in a patient, and treating Alzheimer's Disease by administering peptide inhibitors. Compositions of these peptide inhibitors and pharmaceutically acceptable carriers are also provided, as is a method for identifying inhibitors of GSK-3. The invention further relates to a computer-assisted method of structure based drug design of GSK-3 inhibitors using a three-dimensional structure of a peptide substrate of GSK-3.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:266267 USPATFULL		
TITLE:	Glycogen synthase kinase-3 inhibitors		
INVENTOR(S):	Eldar-Finkelman, Hagit, Shoham, ISRAEL		
PATENT ASSIGNEE(S):	RAMOT UNIVERSITY AUTHORITY FOR APPLIED RESEARCH & INDUSTRIAL DEVELOPMENT LTD., Tel Aviv, ISRAEL, 61392 (non-U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002147146	A1	20021010
APPLICATION INFO.:	US 2001-951902	A1	20010914 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. WO 2001-US123, filed on 3 Jan 2001, UNKNOWN		
	NUMBER	DATE	
PRIORITY INFORMATION:	US 2000-174308P	20000103 (60)	
	US 2000-206115P	20000522 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	BROWDY AND NEIMARK, P.L.L.C., 624 Ninth Street, N.W., Washington, DC, 20001-5303		
NUMBER OF CLAIMS:	37		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	7 Drawing Page(s)		
LINE COUNT:	2508		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 8 OF 25 USPATFULL
TI Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
AB The present invention provides amino acid sequences of peptides that are encoded by genes within the human genome, the phosphatase peptides of the present invention. The present invention specifically provides isolated peptide and nucleic acid molecules, methods of identifying orthologs and paralogs of the phosphatase peptides, and methods of identifying modulators of the phosphatase peptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:251099 USPATFULL
TITLE:	Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
INVENTOR(S):	Wei, Ming-Hui, Germantown, MD, UNITED STATES

Ketchum, Karen A., Germantown, MD, UNITED STATES
Di Francesco, Valentina, Rockville, MD, UNITED STATES
Beasley, Ellen M., Darnestown, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002137042	A1	20020926
APPLICATION INFO.:	US 2001-761640	A1	20010118 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 715177, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: CELERA GENOMICS CORP., ATTN: WAYNE MONTGOMERY, VICE PRES, INTEL PROPERTY, 45 WEST GUDE DRIVE, C2-4#20, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Page(s)
LINE COUNT: 2957
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 9 OF 25 USPATFULL
TI Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
AB The present invention provides amino acid sequences of peptides that are encoded by genes within the human genome, the phosphatase peptides of the present invention. The present invention specifically provides isolated peptide and nucleic acid molecules, methods of identifying orthologs and paralogs of the phosphatase peptides, and methods of identifying modulators of the phosphatase peptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:200019 USPATFULL
TITLE: Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
INVENTOR(S): Wei, Ming-Hui, Germantown, MD, UNITED STATES
Ketchum, Karen A., Germantown, MD, UNITED STATES
Di Francesco, Valentina, Rockville, MD, UNITED STATES
Beasley, Ellen M., Darnestown, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002108133	A1	20020808
APPLICATION INFO.:	US 2001-813319	A1	20010321 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-752820, filed on 3 Jan 2001, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: CELERA Genomics Corporation, 45 West Gude Drive, C2-4#20, Rockville, MD, 20850
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 23 Drawing Page(s)
LINE COUNT: 3018
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 10 OF 25 USPATFULL
TI Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
AB The present invention provides amino acid sequences of peptides that are encoded by genes within the human genome, the phosphatase peptides of the present invention. The present invention specifically provides isolated peptide and nucleic acid molecules, methods of identifying orthologs and paralogs of the phosphatase peptides, and methods of

identifying modulators of the phosphatase peptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:199061 USPATFULL
TITLE:	Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
INVENTOR(S):	Wei, Ming-Hui, Germantown, MD, UNITED STATES Ketchum, Karen A., Germantown, MD, UNITED STATES Di Francesco, Valentina, Rockville, MD, UNITED STATES Beasley, Ellen M., Darnestown, MD, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002107170	A1	20020808	
APPLICATION INFO.:	US 2001-752820	A1	20010103	(9)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	CELERA GENOMICS CORP., ATTN: WAYNE MONTGOMERY, VICE PRES, INTEL PROPERTY, 45 WEST GUDE DRIVE, C2-4#20, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS:	23
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	22 Drawing Page(s)
LINE COUNT:	3003

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 11 OF 25 USPATFULL

TI Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof

AB The present invention provides amino acid sequences of peptides that are encoded by genes within the human genome, the phosphatase peptides of the present invention. The present invention specifically provides isolated peptide and nucleic acid molecules, methods of identifying orthologs and paralogs of the phosphatase peptides, and methods of identifying modulators of the phosphatase peptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:178773 USPATFULL
TITLE:	Isolated human phosphatase proteins, nucleic acid molecules encoding human phosphatase proteins, and uses thereof
INVENTOR(S):	Ye, Jane, Boyds, MD, UNITED STATES Yan, Chunhua, Boyds, MD, UNITED STATES Di Francesco, Valentina, Rockville, MD, UNITED STATES Beasley, Ellen M., Darnestown, MD, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002094561	A1	20020718	
APPLICATION INFO.:	US 2000-738885	A1	20001218	(9)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	CELERA GENOMICS CORP., ATTN: WAYNE MONTGOMERY, VICE PRES, INTEL PROPERTY, 45 WEST GUDE DRIVE, C2-4#20, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS:	23
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	7 Drawing Page(s)
LINE COUNT:	2519

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 12 OF 25 USPATFULL

TI Mammalian protein phosphatases

AB The present invention relates to phosphatase polypeptides, nucleotide sequences encoding the phosphatase polypeptides, as well as various products and methods useful for the diagnosis and treatment of various phosphatase-related diseases and conditions. Through the use of a bioinformatics strategy, mammalian members of the MAP kinase phosphatase PTP's and STP's have been identified and their protein structure predicted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:171954 USPATFULL
TITLE:	Mammalian protein phosphatases
INVENTOR(S):	Plowman, Gregory D., San Carlos, CA, UNITED STATES Martinez, Ricardo, Foster City, CA, UNITED STATES Whyte, David, Belmont, CA, UNITED STATES Manning, Gerard, Menlo Park, CA, UNITED STATES Sudarsanam, Sucha, Greenbrae, CA, UNITED STATES Caenepeel, Sean, Oakland, CA, UNITED STATES Hill, Ron, Burlingame, CA, UNITED STATES Flanagan, Peter, San Francisco, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002090703	A1	20020711	
APPLICATION INFO.:	US 2001-866987	A1	20010530	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-208291P	20000530 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	FOLEY AND LARDNER, SUITE 500, 3000 K STREET NW, WASHINGTON, DC, 20007
NUMBER OF CLAIMS:	32
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	3 Drawing Page(s)
LINE COUNT:	5078

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 13 OF 25 USPATFULL

TI METHOD FOR PREVENTING GASTRITIS USING AMYLIN OR AMYLIN AGONISTS

AB Methods for treating or preventing gastritis or gastric injury are disclosed, comprising administering a therapeutically effective amount of an amylin or an amylin agonist. Methods are also disclosed for the treatment of pain, fever, inflammation, arthritis, hypercoagulability, or other conditions for which a non-steroidal anti-inflammatory drug would be indicated, comprising administering an amylin or amylin agonist in conjunction with administering a therapeutically effective amount of a non-steroidal anti-inflammatory agent. Pharmaceutical compositions comprising an amylin or amylin agonist and a non-steroidal anti-inflammatory drug are also disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:17253 USPATFULL
TITLE:	METHOD FOR PREVENTING GASTRITIS USING AMYLIN OR AMYLIN AGONISTS
INVENTOR(S):	YOUNG, ANDREW A., SAN DIEGO, CA, UNITED STATES GEDULIN, BRONISLAVA, SAN DIEGO, CA, UNITED STATES BEYNON, GARETH W., BRIGHTWELL-CUM SOTWELL, UKRAINE

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002010133	A1	20020124	
APPLICATION INFO.:	US 1997-851965	A1	19970506	(8)

DOCUMENT TYPE:	Utility

FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BRADFORD J. DUFT, ESQ, BROBECK, PHLEGER & HARRISON LLP, 12390 EL CAMINO REAL, SAN DIEGO, CA, 92130
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Page(s)
LINE COUNT: 1152
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 14 OF 25 USPATFULL
TI Clinical use of oxytocin alone or in combination to treat bone disorders
AB The present invention details the use of oxytocin or oxytocin analogs as a novel therapeutic regimen for the treatment of various bone diseases and for assisting in bone remodeling. Oxytocin and oxytocin analogs can be administered alone or in combination with other agents used to treat bone diseases or aid in bone remodeling. In addition, agents which induce endogenous oxytocin release are also contemplated in the present invention for treatment of bone diseases and for assisting in bone remodeling. Diseases and conditions that are contemplated to benefit from the present invention include osteoporosis, osteopenias, bone fractures and bone remodeling surgery.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:235238 USPATFULL
TITLE: Clinical use of oxytocin alone or in combination to treat bone disorders
INVENTOR(S): Copland, III, John A., Houston, TX, United States
Ives, Kirk Lorne, Dickinson, TX, United States
Simmons, David J., St. Louis, MO, United States
Soloff, Melvyn, Galveston, TX, United States
PATENT ASSIGNEE(S): Board of Regents, The University of Texas System, Austin, TX, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6333313	B1	20011225	
APPLICATION INFO.:	US 1999-430114		19991029	(9)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 1998-106134P	19981029	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Criares, Theodore J.
LEGAL REPRESENTATIVE: Fulbright & Jaworski LLP
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 1314
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 15 OF 25 USPATFULL
TI **Receptor** derived peptides as modulators of **receptor** activity
AB Oligopeptides having an amino acid sequence corresponding to a **receptor**'s extracellular domain, and having sequence similarity to regulatory peptides from MHC class I antigens, enhance or replace the physiological response of ligand **binding** to the corresponding **receptor**. The oligopeptides are used in diagnosis and therapy of diseases that involve inadequate or inappropriate **receptor** response as well as in the screening of drug candidates that affect surface expression of receptors. Also useful for drug screening is a modified **receptor** molecule, where the sequence corresponding to the regulatory peptide is modified or deleted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:234974 USPATFULL
TITLE: **Receptor** derived peptides as modulators of **receptor** activity
INVENTOR(S): Olsson, Lennart, Orinda, CA, United States
Naranda, Tatjana, Mountain View, CA, United States
PATENT ASSIGNEE(S): Reception, Inc., Mountain View, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6333031	B1	20011225
APPLICATION INFO.:	US 1998-28937		19980224 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-788820, filed on 23 Jan 1997 Continuation of Ser. No. US 1996-701382, filed on 22 Aug 1996, now patented, Pat. No. US 6004758 Continuation of Ser. No. US 1996-612999, filed on 8 Mar 1996, now patented, Pat. No. US 5952293
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Chan, Christina Y.
ASSISTANT EXAMINER: DiBrino, Marianne
LEGAL REPRESENTATIVE: Rowland, Bertram I.
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 20 Drawing Figure(s); 20 Drawing Page(s)
LINE COUNT: 1909
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 16 OF 25 USPATFULL
TI **Insulin receptor** tyrosine kinase substrate
AB The invention provides a human **insulin receptor** tyrosine kinase substrate (IRS-p53h) and polynucleotides which identify and encode IRS-p53h. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of IRS-p53h.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:117155 USPATFULL
TITLE: **Insulin receptor** tyrosine kinase substrate
INVENTOR(S): Hillman, Jennifer L., Mountain View, CA, United States
Lal, Preeti, Sunnyvale, CA, United States
Shah, Purvi, Sunnyvale, CA, United States
PATENT ASSIGNEE(S): Incyte Genomics, Inc., Palo Alto, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6265550	B1	20010724
APPLICATION INFO.:	US 1999-270117		19990315 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1997-878563, filed on 19 Jun 1997, now patented, Pat. No. US 5891674
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Eyler, Yvonne
ASSISTANT EXAMINER: Lazar-Wesley, Eliane
LEGAL REPRESENTATIVE: Incyte Genomics, Inc.
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 9 Drawing Page(s)
LINE COUNT: 2114

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 17 OF 25 USPATFULL
TI Methods for regulating gastrointestinal motility
AB Methods for treating conditions associated with elevated, inappropriate or undesired post-prandial blood glucose levels are disclosed which comprise administration of an effective amount of an amylin agonist alone or in conjunction with other anti-gastric emptying agents. Methods for reducing gastric motility and delaying gastric emptying for therapeutic and diagnostic purposes are also described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2000:117685 USPATFULL
TITLE:	Methods for regulating gastrointestinal motility
INVENTOR(S):	Kolterman, Orville G., Poway, CA, United States Young, Andrew A., Alpine, CA, United States Rink, Timothy J., La Jolla, CA, United States Brown, Kathleen Ann Keiting, Wake Forest, NC, United States
PATENT ASSIGNEE(S):	Amylin Pharmaceuticals, Inc., San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6114304		20000905
APPLICATION INFO.:	US 1994-302069		19940907 (8)

RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1993-118381, filed on 7 Sep 1993, now abandoned
DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Celsa, Bennett
LEGAL REPRESENTATIVE:	Lyon & Lyon LLP
NUMBER OF CLAIMS:	35
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	17 Drawing Figure(s); 17 Drawing Page(s)
LINE COUNT:	2789

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 18 OF 25 USPATFULL
TI **Insulin receptor** tyrosine kinase substrate
AB The invention provides a human **insulin receptor** tyrosine kinase substrate (IRS-p53h) and polynucleotides which identify and encode IRS-p53h. The invention also provides expression vectors, host cells, agonists, antibodies and antagonists. The invention also provides methods for treating disorders associated with expression of IRS-p53h.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1999:43422 USPATFULL
TITLE:	**Insulin receptor** tyrosine kinase substrate
INVENTOR(S):	Hillman, Jennifer L., Mountain View, CA, United States Lal, Preeti, Sunnyvale, CA, United States Shah, Purvi, Sunnyvale, CA, United States
PATENT ASSIGNEE(S):	Incyte Pharmaceuticals, Inc., Palo Alto, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5891674		19990406
APPLICATION INFO.:	US 1997-878563		19970619 (8)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Feisee, Lila

ASSISTANT EXAMINER: Lazar-Wesley, Eliane
LEGAL REPRESENTATIVE: Price, Esq., Leanne C., Billings, Esq., Lucy J.Incyte Pharmaceuticals, Inc.
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 9 Drawing Page(s)
LINE COUNT: 2207
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 19 OF 25 USPATFULL
TI Methods for regulating gastrointestinal motility
AB Methods for treating conditions associated with elevated, inappropriate or undesired post-prandial blood glucose levels are disclosed which comprise administration of an effective amount of an amylin agonist alone or in conjunction with other anti-gastric emptying agents. Methods for reducing gastric motility and delaying gastric emptying for therapeutic and diagnostic purposes are also described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:98885 USPATFULL
TITLE: Methods for regulating gastrointestinal motility
INVENTOR(S): Kolterman, Orville G., Poway, CA, United States
Rink, Timothy J., La Jolla, CA, United States
PATENT ASSIGNEE(S): Amylin Pharmaceuticals Inc., San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5795861		19980818
APPLICATION INFO.:	US 1995-471675		19950605 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1994-302069, filed on 7 Sep 1994 which is a continuation-in-part of Ser. No. US 1993-118381, filed on 7 Sep 1993
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Hill, Jr., Robert J.
ASSISTANT EXAMINER: Harle, Jennifer
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 26 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 1905
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 20 OF 25 USPATFULL
TI Appetite regulating compositions
AB Compositions and methods for reducing food intake, suppressing appetite and controlling body weight are provided. Such compositions may include an amylin agonist and a CCK agonist or a hybrid peptide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:39498 USPATFULL
TITLE: Appetite regulating compositions
INVENTOR(S): Rink, Timothy J., 6041 Camino De La Costa, La Jolla, CA, United States 92037
Young, Andrew A., 510 Josh Way, Alpine, CA, United States 91901
Beeley, Nigel Robert Arnold, 227 Loma Corta Dr., Solana Beach, CA, United States 92037
Prickett, Kathryn S., 7612 Trailbrush Ter., San Diego, CA, United States 92126

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5739106		19980414

APPLICATION INFO.: US 1995-477727 19950607 (8)
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Tsang, Cecilia J.
ASSISTANT EXAMINER: Harle, Jennifer
LEGAL REPRESENTATIVE: Lyon & Lyon LLP
NUMBER OF CLAIMS: 85
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 2214
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 21 OF 25 USPATFULL
TI Methods and compositions for treating pain with amylin or agonists thereof
AB Methods for treating pain are disclosed which comprise administration of a therapeutically effective amount of an amylin or an amylin agonist alone or in conjunction with a narcotic analgesic or other pain relief agent.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 97:94212 USPATFULL
TITLE: Methods and compositions for treating pain with amylin or agonists thereof
INVENTOR(S): Young, Andrew A., San Diego, CA, United States
PATENT ASSIGNEE(S): Amylin Pharmaceuticals, Inc., San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5677279		19971014	
APPLICATION INFO.:	US 1996-767169		19961216	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jarvis, William R.A.
LEGAL REPRESENTATIVE: Lyon & Lyon
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 10 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT: 1264
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 22 OF 25 USPATFULL
TI Methods for treating renin-related disorders with amylin antagonists
AB Methods for treating conditions associated with elevated, inappropriate or undesired renin activity are disclosed which comprise administration of an effective amount of any amylin **antagonist** alone or in conjunction with other anti-hypertensive agents. Methods for screening for and/or evaluating anti-renin amylin antagonists are also described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 94:113002 USPATFULL
TITLE: Methods for treating renin-related disorders with amylin antagonists
INVENTOR(S): Young, Andrew A., San Diego, CA, United States
Rink, Timothy J., La Jolla, CA, United States
PATENT ASSIGNEE(S): Amylin Pharmaceuticals, Inc., San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5376638		19941227	
APPLICATION INFO.:	US 1992-939106		19920901	(7)

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted
PRIMARY EXAMINER: Lee, Lester L.
LEGAL REPRESENTATIVE: Lyon & Lyon
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 1037
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 23 OF 25 USPATFULL
TI Method and composition for calcium **binding**, translocation and mediating
AB A method, and mediating agents are provided for mediating the physiological effects of hormones, neurotransmitters, calcium-channel antagonists, chemotactic peptides or chemotactic proteins. The mediating agents provided herein are bioactive conformations of peptide hormones, neurotransmitters, calcium-channel **antagonist** drugs or chemotatic peptides, or analogues, agonists or antagonists thereof, or synthetic analogue substances, having Ca.sup.2+ and/or Mg.sup.2+ ions optimally and optimally-conformationally bound to the respective **compound**. The Ca.sup.2+ **antagonist**/agonist may bind Mg.sup.2+ and thus may also block calcium-channel. In the one method, the concentration of intracellular Ca.sup.2+ is raised by means of such mediating agents. A second method is also provided for delivering Ca.sup.2+ and/or Mg.sup.2+ to a membrane-bound **receptor** by transporting Ca.sup.2+ and/or Mg.sup.2+ through a cell membrane using the above-described mediating agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 92:101020 USPATFULL
TITLE: Method and composition for calcium **binding**, translocation and mediating
INVENTOR(S): Ananthanarayanan, V. S., Ancaster, Canada
PATENT ASSIGNEE(S): Seabright Corporation Limited, Newfoundland, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5169865		19921208	
APPLICATION INFO.:	US 1989-323421		19890314	(7)

	NUMBER	DATE
PRIORITY INFORMATION:	CA 1988-572968	19880725

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Lee, Lester L.
ASSISTANT EXAMINER: Kraus, E. J.
LEGAL REPRESENTATIVE: Cooper, Iver P.
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Figure(s); 3 Drawing Page(s)
LINE COUNT: 575
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 24 OF 25 USPATFULL
TI Composite **binding** site drugs
AB Methods and compositions are provided for regulating surface membrane **receptor** response by providing novel proteinaceous compositions to bind to two separate sites of a surface membrane **receptor**, the **binding** site of the **receptor** and an allosteric site, which binds to a sequence of a Class I MHC antigen. The resulting products may act as agonist or **antagonist** to the normal function of the **receptor** and find use in the control of

physiological processes.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	91:102206 USPATFULL		
TITLE:	Composite **binding** site drugs		
INVENTOR(S):	Olsson, Lennart, Orinda, CA, United States		
PATENT ASSIGNEE(S):	Receptron, Concord, CA, United States (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5073540		19911217
APPLICATION INFO.:	US 1989-351764		19890515 (7)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Moskowitz, Margaret		
ASSISTANT EXAMINER:	Feisee, Lila		
LEGAL REPRESENTATIVE:	Rowland, Bertram I.		
NUMBER OF CLAIMS:	6		
EXEMPLARY CLAIM:	1		
LINE COUNT:	837		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L23 ANSWER 25 OF 25 USPATFULL

TI **Insulin-binding** peptides and uses thereof

AB The present invention concerns a novel class of peptides which specifically bind **insulin**. The diverse uses of these peptides relate to **insulin**, **insulin** action and the cellular **receptor** for **insulin**. The most preferred peptide of the invention is the hexapeptide-NH.sub.2 -cys-val-glu-glu-ala-ser-COOH which has marked affinity for the **insulin** B-chain.

Antibodies having specific **binding** affinity for peptides of the invention have identified and forseen uses. Such antibodies specifically directed toward a preferred peptide of the invention comprising cys-val-glu-glu-ala-ser represent a further preferred aspect of antibody-related aspects of the present invention.

In one significant aspect, the present invention involves a method for determining **insulin** levels in a biological fluid. A further aspect of the present invention involves a process for measuring **insulin receptor** sites involving the **binding** of antibodies to cells or cell fractions.

The present invention further comprises a method for decreasing the amount of biologically active **insulin** present in a biological fluid such as the blood of an animal. This method involves parenterally administering an amount of peptide of the invention in free or carrier-conjugated form.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	89:100687 USPATFULL		
TITLE:	**Insulin-binding** peptides and uses thereof		
INVENTOR(S):	Knutson, Victoria P., Pearland, TX, United States		
PATENT ASSIGNEE(S):	Board of Regents, University of Texas System, Austin, TX, United States (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 4888414		19891219
APPLICATION INFO.:	US 1987-36215		19870409 (7)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		